

Plasma free of factors II, VII, IX, and X

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Letter to the Editor

Plasma Free of Factors II, VII, IX, and X Need for High Speed Centrifugation after $Al(OH)_3$ Absorption



F. K. SCHATTAUER VERLAG · STUTTGART-NEW YORK

Dear Madam,

29th December, 1972

The procedure to remove the vitamin K-dependent clotting factors from citrated plasma (either normal or from patients treated with oral anticoagulants) has been well standardized with respect to the type of $\text{Al}(\text{OH})_3$ to be used (a preparation made by "British Drug Houses" has been used, 100 g of which being suspended in 400 ml of distilled water), the time of incubation and the temperature at which adsorption takes place (Biggs, 1972). The importance of standardizing the centrifugation step as well, is insufficiently realized, however.

An apparently clear supernatant is obtained after a 2.500–3.000 rpm. run for 10 min. at room temperature, which suggests complete removal of $\text{Al}(\text{OH})_3$. However, if the supernatant thus obtained (low-speed supernatant) is used for the construction of a thrombotest reference curve (Fig. 1), values are obtained, which are not only largely

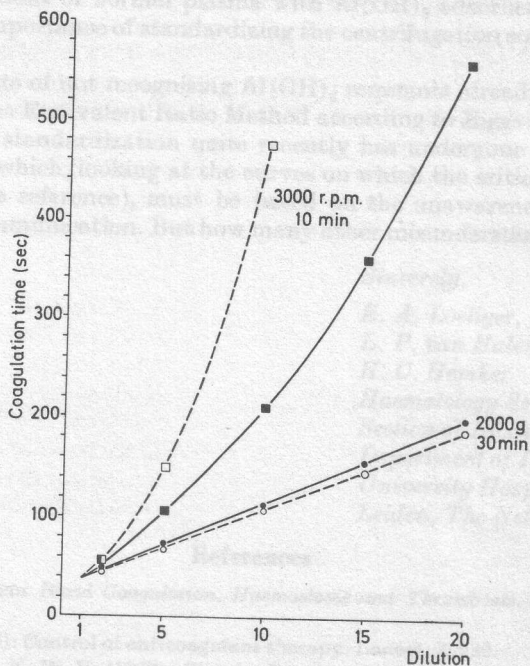


Fig. 1 Correlation between coagulation time (Thrombotest) and dilution of normal plasma in $\text{Al}(\text{OH})_3$ -adsorbed plasma. 1 = undiluted normal plasma; 2 = 1/2 dilution etc. The lower curves are obtained from normal plasma diluted in high-speed supernatant, ●—● with not incubated dilutions, and ○—○ with dilutions incubated for 3 hours at room temperature. The upper curves are obtained from dilutions in low-speed supernatant; ■—■ immediately, and □—□ 3 hours after dilutions had been prepared.

different from those obtained with saline or buffer (the reference curve given by the producer of thrombotest), but which are also far from stable; coagulation times of the diluted plasma samples are considerably longer after three hours incubation at room temperature than immediately after the preparation of the dilutions. In contrast, with supernatant obtained after centrifugation at 20.000 g for 30 min. at 4°C (high-speed supernatant) the curve parallels the producer's saline or buffer curve, independent of the time of incubation of the dilutions. High-speed supernatant, therefore, is the only preparation suitable for use as a diluant in the constructions of reference curves.

The differences in coagulation times between low-speed and high-speed supernatant seen in Fig. 1 are most easily explained as caused by remnants of $\text{Al}(\text{OH})_3$ still present in the low-speed supernatant and adsorbing, at a relatively low rate, factors II, VII, IX, and X from the diluted normal plasma. Indeed a small pellet of $\text{Al}(\text{OH})_3$ can be found at the bottom of the tube after high-speed centrifugation of the low-speed supernatant.

When reference curves of thromboplastins other than Thrombotest are considered, the influence of $\text{Al}(\text{OH})_3$ remnants is much less obvious. This is caused partly by the lower sensitivity of most of the other thromboplastins, partly by a factor VII- or factor X-like activity often present in ordinary tissue thromboplastins. This might be the reason why the effect has escaped the observer's eye for such a long time.

Since many investigators still refer to percentages obtained from reference curves obtained from dilutions of normal plasma with $\text{Al}(\text{OH})_3$ adsorbed plasma (Leading article, 1969), the importance of standardizing the centrifugation step at a high g value is obvious.

The adverse effects of not recognising $\text{Al}(\text{OH})_3$ remnants already haunt the literature: for instance the Equivalent Ratio Method according to Biggs and Denson (1967) for thromboplastin standardization quite recently has undergone a criticism (Littel and Ratnoff, 1969) which, looking at the curves on which the criticism is based (page 143 and 144 of this reference), must be based on the unawareness of the artefact described in this communication. But how many other misunderstandings do exist?

Sincerely,

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